

Elevated levels of peripheral-blood, naturally occurring aliphatic polyamines in bronchial asthmatic patients with active symptoms

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The levels of peripheral-blood, naturally occurring aliphatic polyamines, such as putrescine, spermidine, and spermine, from 21 bronchial asthmatic patients (11 atopics and 10 nonatopics) were measured by postcolumn derivatization high-performance liquid chromatography analysis. None of the patients, except the 44-year-old woman in the case report below, were given prednisolone, and they were instructed to take only regular medication during the tests. Blood was drawn from the patients in a fasting state, and the polyamine levels were compared between the times when they were free of asthmatic symptoms and when they had mild spontaneous attacks. Nine (5 atopics and 4 nonatopics), 6 (3 atopics and 3 nonatopics), and 4 (3 atopics and 1 nonatopic) out of 20 patients, when they had relatively mild asthmatic attacks, showed higher putrescine, spermidine, and spermine levels, respectively, than those of normal healthy control subjects. The levels of peripheral blood polyamines from a 44-year-old atopic bronchial asthmatic woman, who was admitted to the hospital with severe asthmatic attacks, were measured serially, and the putrescine and spermidine levels were found to be elevated during the asthmatic attacks, returning to normal levels in parallel with the clinical course. These data may suggest a role for naturally occurring aliphatic polyamines in bronchial asthma.

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Aliphatic polyamines are naturally occurring cations, and their ubiquitous distribution and the complexity of the mechanisms that regulate their intracellular concentrations suggest important roles for these compounds in normal cellular growth and differentiation (4, 8). Recent investigations from our laboratory indicate that naturally occurring aliphatic polyamines, such as spermine and spermidine, stimulate the phosphatidylinositol kinase that catalyzes the formation of phosphatidylinositol 4-phosphate from endogenous phosphatidylinositol in rat peritoneal mast cell granules (6). They also induce a significant histamine release from rat peritoneal mast cells (7), suggesting possible involvement of polyamines in allergic reactions. These findings made us investigate the levels of peripheral-blood, naturally occurring aliphatic polyamines in bronchial asthmatic patients, and the present study indicates a possible role for the polyamines in bronchial asthma.

Subjects and methods

Reagents

Reagents used in the present study and their sources were as follows: putrescine hydrochloride, spermidine hydrochloride, spermine hydrochloride (Sigma Chemical Co., St Louis, MO, USA); orthophthaldialdehyde (OPA), sulfosalicylic acid, 2-mercaptoethanol, sodium acetate, sodium chloride, hydrochloric acid, acetonitrile (Kokusan Chemical Works, Tokyo, Japan). All other chemicals were analytic grade.

Subjects

Subjects were divided into normal healthy subjects and bronchial asthmatic patients. None had histories of smoking. The control group comprised 56 subjects (26 males and 30 females) in the age range 19–43 years (average age 28 years), with

normal pulmonary function (FEV_1 was 98 ± 5 [mean $\pm$ SEM] of predicted) and no personal and/or family history of allergic disorders. The diagnosis of bronchial asthma in "asthmatic patients" was based on routine clinical criteria, including complete history, physical examination, blood and urine analysis, examination of sputa for eosinophilic leukocytes, chest roentgenography, pulmonary function tests, and intradermal skin tests with inhalant allergen extracts; all subjects showed evidence of variable bronchial obstruction.

The asthmatic group comprised 21 persons (10 male and 11 female) in the age range 19–41 years (average age 29.5 years). Asthma was classified among the asthmatic patients as atopic in 11 persons (5 male and 6 female) in the age range 19–41 years (average age 29 years), and nonatopic in 10 persons (5 male and 5 female) in the age range 23–40 years (average age 30.2 years). The term "atopic" refers to patients with well-defined allergies to inhalant antigens such as house dust.

There were no significant age differences between the groups. All had had at least one episode of relatively mild symptoms (FEV_1 was 67.8 ± 6.9 [mean $\pm$ SEM] of predicted) within 3 months before the study. The asthmatic subjects had been controlled by disodium cromoglycate 40–80 mg/d and β -adrenergic bronchodilators, such as mabuterol hydrochloride 50–100 μ g/d or clenbuterol hydrochloride 10–20 μ g/d and theophylline 400–600 mg/d. No patients, except a 44-year-old woman to be reported below, were given prednisolone.

Unless otherwise indicated, the asthmatic subjects were free of symptoms for the 2 weeks preceding the initial blood test and had no detectable symptoms at the time of the initial study. In addition to this test, blood was drawn from the same patients when they had active asthmatic symptoms spontaneously, such as wheezing and breathlessness on moderate exercise (FEV_1 was 60.4 ± 6.8 [mean $\pm$ SEM] % of predicted). They had been instructed to take only regular medication, as described above, before coming to the hospital. Patients with chronic bronchitis and emphysema without recurrent, reversible bronchospasm were excluded from the study. The asthmatics and normal healthy subjects were all volunteers and were within 10% of their ideal weight.

Plan of experiment

Subjects gave informed consent and came to the laboratory in a fasting state, having been instructed to take regular medication. Each subject was placed in bed and blood was drawn from 0830 to 0900. One-half milliliter of heparinized whole blood was mixed with 1 ml of double-glass-distilled water and

1 ml of 5% (w/v) sulfosalicylic acid by inversion five times with minimal foaming. After standing for 15 min at room temperature and centrifugation at 1000 g for 15 min, the supernatant was filtered through a 0.45 μ m microfilter (Toyo Roshi Co., Tokyo, Japan) and loaded on the HPLC column, as described below. Treatment of standard polyamine solutions by this method resulted in recoveries of more than 94%.

Chromatographic system

Postcolumn derivatization high-performance liquid chromatography (HPLC) analysis was performed by the method described by Takagi et al. (13). Fig. 1 shows a flow diagram of the automatic polyamine analyzer which was operated in our laboratory. Two high-pressure piston pumps (Senshyu Scientific Industry Co., Tokyo, Japan) served to pump the eluent and OPA solution. The column eluent was pumped through an automated sample injector (WISP 710W, Millipore Corporation, Milford, MA, USA). A jacketed, stainless-steel column, 3.0 $\times$ 0.46 cm internal diameter, was used, slurry-packed with CK-10U cation-exchange resin with a mean particle size of 5.0 ± 0.5 (mean $\pm$ SD) μ m (Mitsubishi Kasei Co., Tokyo, Japan). The column temperature was kept at 60°C with a constant-temperature circulator. The reservoir of the OPA solution was kept in a refrigerator at 4°C to prevent degradation of the reagent. All buffer solutions were delivered by a GPA-40 gradient programmer (Japan Spectroscopic Co., Tokyo, Japan). A JASCO FP-110 fluorescence spectrophotometer (Japan Spec-

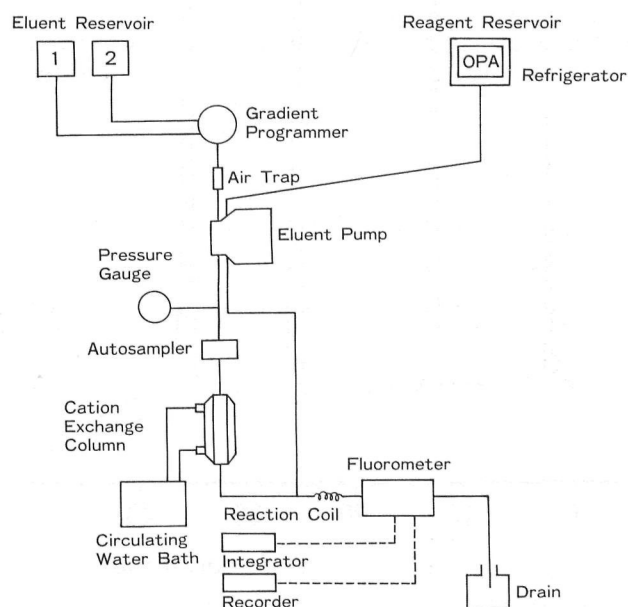


Fig. 1. Flow diagram of the polyamine analyzer.

troscopic Co., Tokyo, Japan) was used to detect the fluorescent polyamine derivatives produced by the reaction with OPA-2-mercaptoethanol. The fluorescence setting was activated at 340 nm, and emission was measured at 455 nm. The fluorometric signal was recorded by a SEKONIC SS-25F recorder (Japan Spectroscopic Co., Tokyo, Japan) at a chart speed of 15 cm/h. Peak areas were determined with a digital integrator (Chromatopac C-R1B, Shimadzu Seisakusho Co., Kyoto, Japan).

Eluent and reagent solutions

All reagents were of analytic grade, made up in double-glass-distilled water. All aqueous reagents were filtered through a 0.45 µm microfilter and degassed. Standard polyamine solutions were dis-

solved in 0.1 mol/l hydrochloric acid with putrescine, spermidine, and spermine, each at 10 nmol per 100 ml; these solutions could be kept at 4°C for 6 months without any degradation. The OPA solution was made with 0.8 g/l OPA dissolved in a 1.5 mol/l potassium borate solution, pH 10.5, and 1 ml/l 2-mercaptoethanol. Polyamines were eluted with a gradient of the following two buffers. A) 0.9 mol/l NaCl in 0.28 mol/l sodium acetate, pH 4.9, with 5% (v/v) acetonitrile; B) 2.5 mol/l NaCl in 0.28 mol/l sodium acetate, pH 4.9, with 5% (v/v) acetonitrile. The column was equilibrated at 60°C with buffer A for 25 min. Then 50 µl of the sample was loaded onto the column, and elution was achieved at a flow rate of 0.7 ml/min by a linear gradient starting with 100% buffer A and leading within 15 min to 100% buffer B. Next the program was held for 13 min at a column inlet pressure of 80–100 kg/cm². The OPA solution was pumped into the reaction coil at a flow rate of 0.1 ml/min.

Results

Chromatogram of polyamines

Fig. 2 shows a chromatogram of a standard mixture of polyamines, each at a concentration of 10 nmol/ml. The reproducibility of the analytic system was evaluated from three chromatograms of standard samples. The retention times were as follows: putrescine, 11.13 ± 0.12 (mean $\pm$ SD) min; spermidine, 23.56 ± 0.38 min; and spermine, 32.83 ± 0.36 min. The retention time of each polyamine compound showed a relative standard deviation of $<1.7\%$. The deviation of the peak heights was within 1.6% for all the compounds. The detection limit was 0.4 nmol/ml.

Peripheral blood polyamine levels from normal healthy subjects

The levels of peripheral blood polyamines from normal healthy subjects were less than 0.4 nmol/ml of putrescine, 9.67 ± 1.31 (mean $\pm$ SD) nmol/ml of spermidine with the range from 5.6 nmol/ml to 16.7 nmol/ml, and 5.87 ± 1.40 nmol/ml of spermine with the range from 3.0 nmol/ml to 11.5 nmol/ml.

Peripheral blood polyamine levels from asthmatic patients with and without mild spontaneous attacks

As shown in Fig. 3, the levels of peripheral blood polyamines were compared among the same patients between the times when they had mild spontaneous attacks and when they did not. All patients showed normal putrescine levels when they were free of asthmatic symptoms. The levels of peripheral blood polyamines from some of the bronchial asthmatic patients with mild spontaneous attacks were shown

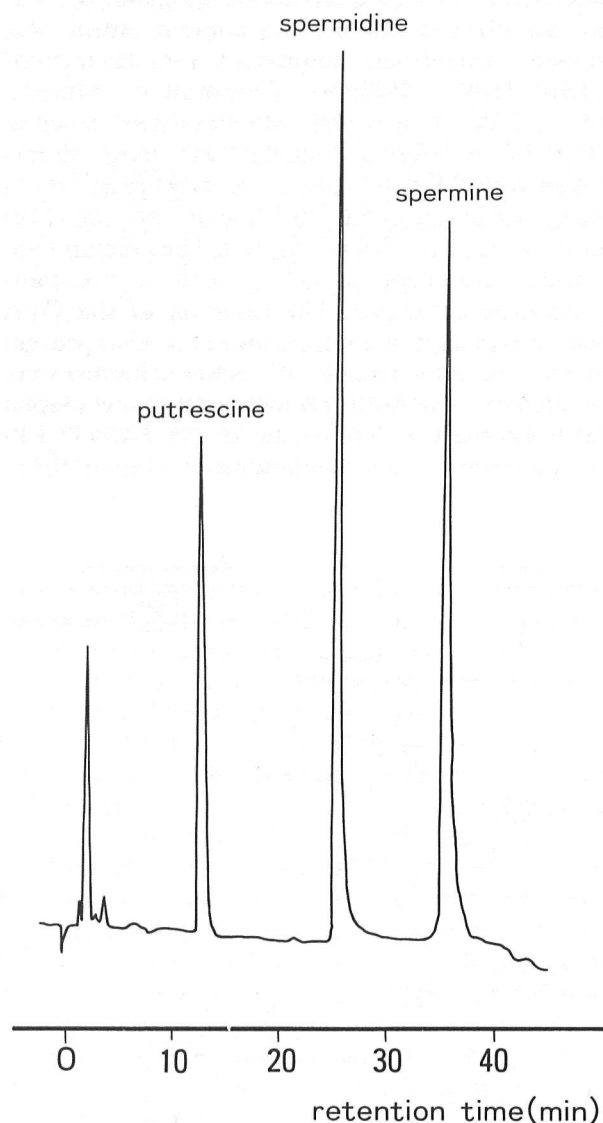


Fig. 2. Chromatogram of a standard mixture of polyamines, each at a concentration of 10 nmol/ml.

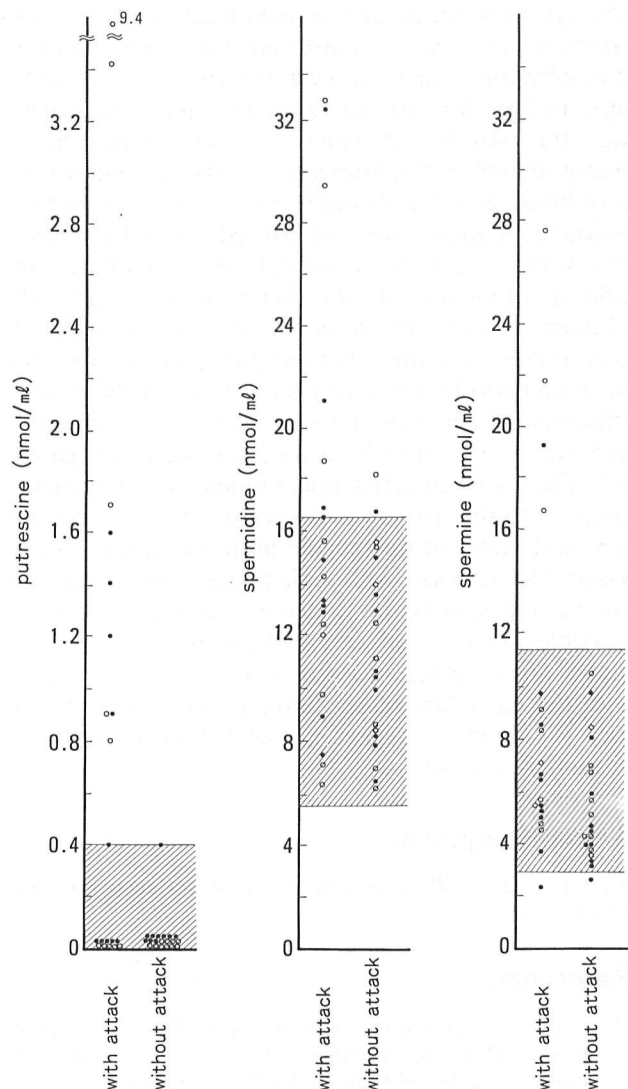


Fig. 3. Comparison of peripheral blood polyamine levels among the same bronchial asthmatic patients between the times when they were free of the asthmatic symptoms and when they had mild, spontaneous attacks. Shaded areas indicate the ranges of each polyamine level from normal healthy control subjects. ○: atopic bronchial asthmatics; ●: nonatopic bronchial asthmatics.

to be elevated irrespective of their type. Specifically, 9 (5 atopics and 4 nonatopics) out of 20 patients showed elevated putrescine levels when they had mild spontaneous attacks. Six (3 atopics and 3 nonatopics) and 4 (3 atopics and 1 nonatopic) patients with the attacks showed higher spermidine and spermine levels, respectively, than the range from normal healthy control subjects. The levels of peripheral blood putrescine and spermidine were constantly elevated in the bronchial asthmatic patients whose peripheral blood spermine levels were elevated.

Case report (Fig. 4)

A 44-year-old woman who had been diagnosed in 1969 as having the atopic, perennial type of bronchial asthma was admitted to our hospital on 13 July 1989 with severe asthmatic attacks. Her condition improved remarkably with adrenaline, aminophylline, and hydrocortisone treatment. After 20 July, she was treated only with oral medicines and was discharged on 4 August 1989. During this episode the levels of peripheral blood polyamines were measured on 5, 13, 22 July and 2 August, and the putrescine and spermidine levels were found to be elevated only during the asthmatic attacks, falling to the levels from normal healthy subjects in parallel with the clinical course.

Discussion

Polyamines are ubiquitous in animal cells, tissues, and extracellular fluids, and are recognized as important regulators of cell functions such as cell differentiation and proliferation (4, 8, 11). They have been shown to interact with polyphosphoinositides (12) and cause an increase in the concentration of polyphosphoinositides in the plasma membranes (14).

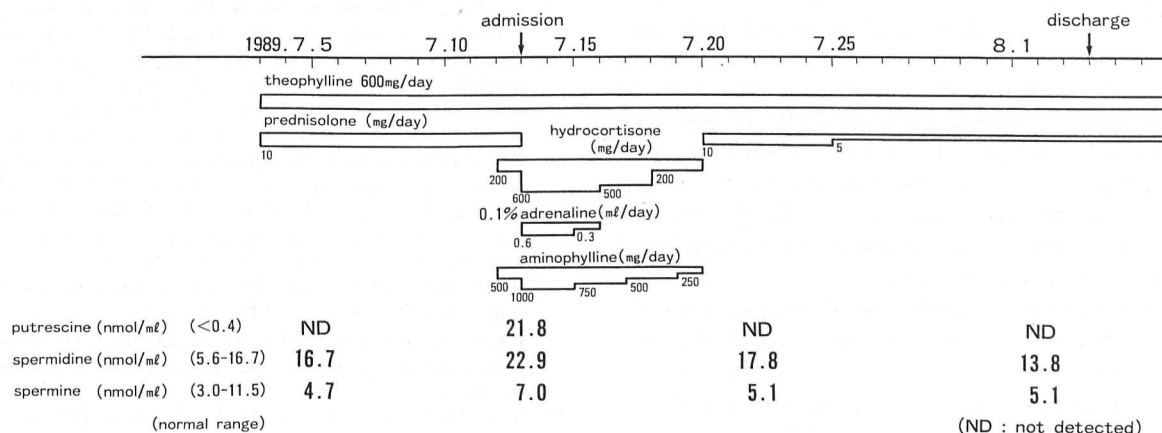


Fig. 4. Clinical course of a 44-year-old woman who was admitted to the hospital with severe asthmatic attacks.

Recent investigations from our laboratory indicate the presence of increased polyphosphoinositide metabolism in activated rat mast cells (5). Moreover, it has been demonstrated that naturally occurring aliphatic polyamines, such as spermine and spermidine, stimulate the phosphorylation of phosphatidylinositol in rat mast cell granules (6) and induce histamine release from rat mast cells (7). Interestingly, spermine at a concentration of 0.1 mmol/l, which in itself could not cause a significant histamine release, showed a tendency to enhance anti-IgE-induced histamine release from mast cells (7). These findings, taken together with the widely accepted idea that mast cells are involved mainly in the immediate asthmatic response (3), led us to investigate the levels of peripheral blood polyamines among the same bronchial asthmatic patients with and without attacks.

Unfortunately, the bronchial allergen challenge test could not be done in the present study because informed consent about the challenge test was not given by the patients. Therefore, the levels of peripheral blood polyamines were compared among the same patients between the times when they were free of the asthmatic symptoms and when they had mild spontaneous attacks. Although this is much less convincing than clear-cut changes in response to allergen challenge, the patients with spontaneous attacks were medicated only by the regular agents, suggesting that the levels of peripheral blood polyamines could be mainly affected by the attacks.

The peripheral blood polyamine levels of a 44-year-old woman who was admitted to our hospital with severe asthmatic attacks were measured serially. The putrescine and spermidine levels were found to be more elevated during the asthmatic attacks than the control levels of normal healthy subjects and to fall to the normal control levels in parallel with the clinical course. Even though the fall of the polyamine levels could have been due to the treatment rather than to improvement in the asthma, there was no doubt that the polyamine levels were elevated when the patient had a severe asthmatic attack.

Besides tumors (4), increased concentrations of the polyamines in human body fluids have been documented in several other pathologic conditions, such as cystic fibrosis (1), psoriasis (9), chronic renal failure (10), polycythemia vera (2), etc. In the present study we demonstrated that the levels of peripheral blood polyamines from some bronchial asthmatic patients were elevated during mild spontaneous attacks irrespective of their type. Because of the limited number of bronchial asthmatic patients investigated, clear-cut differences between the patients whose polyamine levels were elevated and those whose were not could not be obtained. No special

findings were made in the asthmatic subjects with raised levels of polyamines, and there were no clinical symptoms such as skin blotchiness, hypotension, or headache in the asthmatic subjects, revealing no strong evidence of increased mast degranulation in the subjects. In the present study, peripheral blood polyamine levels from bronchial asthmatic patients were measured when they were free of the asthmatic symptoms and when they had mild spontaneous attacks; therefore, it is unknown whether or not the patients with raised levels of polyamines may show the elevated polyamines levels in all periods of the attacks. Moreover, the direct relationship between the levels of the polyamines and the severity of asthma could not be investigated yet. Thus, the elevated polyamines may be consequential to the presence of severe asthmatic condition and may not be specific findings related to the asthmatic attacks. Although further investigations are needed, this is the first paper which shows the possible presence of elevated levels of peripheral blood polyamines, and our results, taken together with the early laboratory findings from our laboratory (6, 7), may suggest a role for polyamines in bronchial asthma.

Acknowledgment

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